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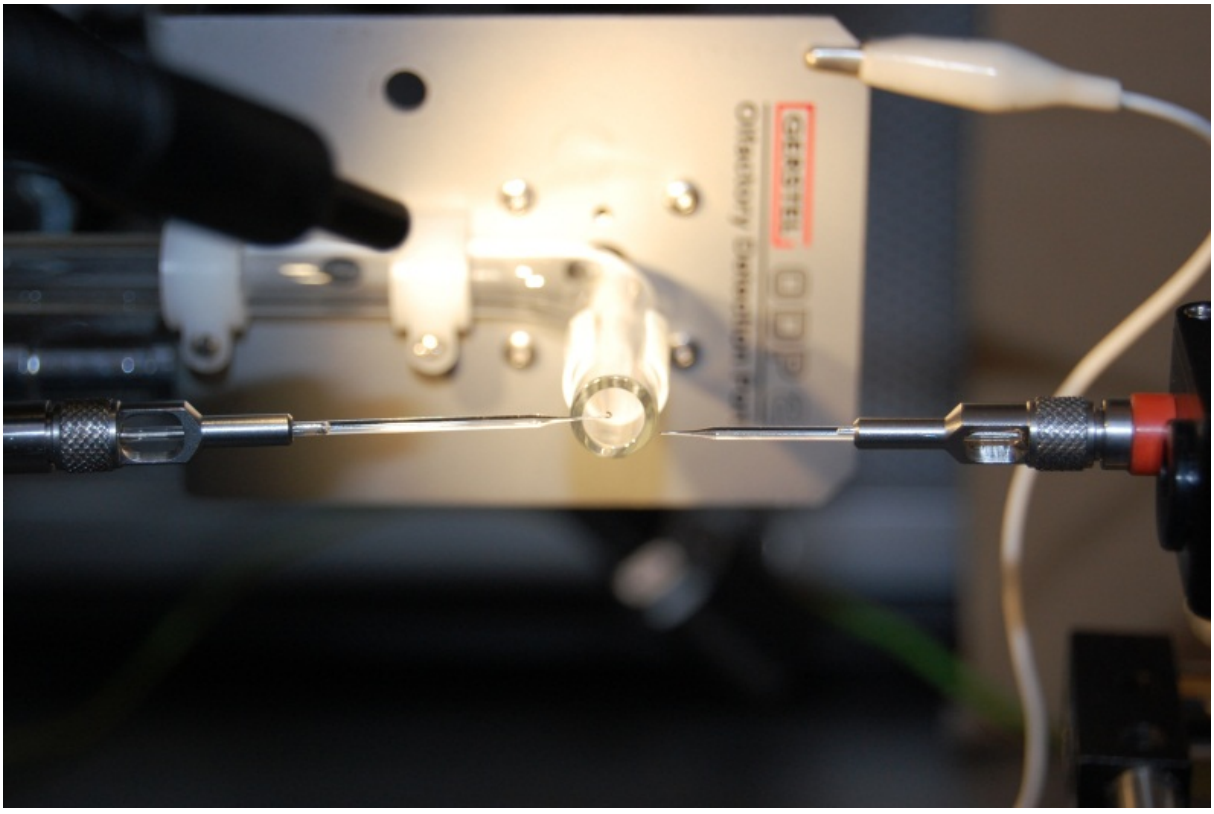

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Skillnader i värdväxtpreferenser och luktfysiologi mellan populationer av flyt *Spodoptera littoralis* etablerad i Egypten och Benin

Louise Wyke

Handledare: *Mattias Larsson*, SLU, Institutionen för växtskyddsbiologi

Btr handledare: *Peter Anderson*, SLU, Institutionen för växtskyddsbiologi

Examinator: *Håkan Asp*, SLU, Institutionen för biosystem och teknologi

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Abstract

This study was made to compare and distinguish any differences in olfactory recognition and host plant adaptation between two strains of cotton leaf worm, *Spodoptera littoralis*. The populations used were established in Egypt and Benin, Africa. The documentation consisted of information about (1) each of the strains development through the larval stage up to pupation on diets of cotton, clover and cowpea. (2) Their oviposition preferences as adults between cotton, clover, cowpea, maize and cabbage. (3) Electroantennographic recordings to quantify any possible responses to plant volatiles.

Throughout the diet experiments the larvae from the Benin strain showed significantly a better development between artificial, clover, cowpea and cotton respectively, while the Egypt strain had significance between artificial, cotton and cowpea/clover as diet.

Also noted was how the strains substantial development of weight differentiated if fed the cotton diet, followed by the cowpea diet showing that the Benin strain would develop a higher body mass on a cotton diet as well as on a cowpea diet.

This trend was however switched once the pupal stage was reached, where the Egyptian strain contained a significantly greater body mass than the Benin strain on the artificial and the Benin strain a greater on cowpea, which suggests that in the end, the Benin strain develops significantly better on cowpea while the Egypt strain develops better on an artificial diet.

During the oviposition experiment that both stains showed a clear difference in hierarchy of host plants considered suitable for oviposition, where the Egyptian strain is significantly more attracted to clover than the Benin strain.

Also showed with electroantennographic recordings and significant values was the higher sensitivity the Egypt strain had towards (E)2-hexenal, (E/Z)-b-ocimene, (Z)-3-hexenyl acetate, Nonanal, (-)-linalool and β-myrcene when compared to the responses from the Benin strain.

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Introduction

Plants and insects share a long co-evolutionary history. Herbivorous insects have been adapting to virtually all plant groups, and during the last 130 million years they stand for the greatest evolutionary radiation together with angiosperms, driving adaptations and counter-adaptations in both feeding and pollination strategies (Crawley, 1989; Soltis *et al.,* 2008; Janz and Nylin 2008). Both broad and restricted feeding patterns have evolved multiple times in different lineages during this period (Janz and Nylin 2008), and both types are assumed to involve specific adaptations but for different life styles.

The plasticity exhibited by generalist herbivorous insects, feeding on a wide range of different host plants that may sometimes span several different families, allows them to exploit a broader range of resources or move to a less stressful or harmful environment when necessary. However, a generalist life style is also associated with specific costs, such as the need to consider far more factors before reaching a decision, leading to prolonged decision time, imperfect decision making, and outright ovipositon mistakes. From a physiological point of view, polyphagy is often expected to result in imperfect adaptation to and utilisation of any of the hosts (Bearnays, 2001). A great majority of herbivorous insects (about 80-90%) are specialized feeders on single or a very limited range of plant species, implying that specialization has greater overall advantages than being polyphagus (Bernays & Graham, 1988; Schoonhoven *et al.* 2005).

Physiological ability to utilize different plant species during the larval stage is one of the fundamental aspects of the host adaptations shaped by evolution. Factors including secondary chemicals such as toxins and constitutive chemical defences, physical defences including tough protective structures and trichomes, and the nutritional value of a plant, are all key components causing strong aggregate selection pressures for physiological adaptations in larvae (Schoonhoven *et al.,* 2005). Among other factors affecting larval survival, directly or indirectly related to the plant itself, are the presence of competitors which may affect the level of induced defences of the plant. Larval mortality from predators and parasitoids is also often directly related to an interaction between the host plant itself, and the presence of other conspecifics or heterospecifics that influence the attraction of these predators and parasitoids to the host plant (Schoonhoven *et al.,* 2005).

For many or most herbivorous insects, development and survival of the offspring is entirely dependent on females finding a suitable host for oviposition. This means ensuring a nutritious food source and good protection for the offspring a priority for adults when choosing an oviposition site. The host plant selection method of herbivorous insects involves the processing

of different sensory cues, where odours often play a major role, some emitted from the plants themselves and others by organisms associated with it. Host plants usually appear to be identified by means of their unique odour blends, involving the presence and concentration levels of a varying number of key components. Perceived out of its context, many of the compounds may not even be recognized or associated with this specific host plant (Bruce & Pickett, 2011). Final oviposition decisions are usually based on an interaction between positive and negative chemical cues (Renwick, 1989).

Adaptation to new hosts is common in insects, and may be a driving force in host race formation and speciation. For example, in the apple maggot fly, *Rhagoletis pomonella*, mating takes place on the host, and host preferences thus also affect mating preferences (Feder, 1998). Their natural host is hawthorn, *Crataegus* L., but over the years the fly slowly started infesting other fruits which resulted in a well-established population on the domesticated apple, *Malus pumila* L., after it was introduced during the mid-1800 in America (Bush 1993). Today the two populations coexist on the same continent and represent a species that have evolved different mate selection systems sufficient to maintain these genetic distinctions (Diehl and Bush, 1984). This evolution is the direct outcome of adaptation in a relatively small area and short amount of time to a new host in the absence of any physical barriers hindering the development (Bush 1993). The *S. littoralis* high plasticity might in the same way cause an adaptation over the African continent, based on odour detection.

The sensitive odour detection systems of moths and most other insects, which are a requirement for survival and reproduction, are mainly used when locating a mate, food or oviposition sites. In either case, the semiochemicals used, the odours capable of carrying a message, are detected by the antennae, the main olfactory organs, where they are transformed into an electrical signal interpreted by the central nervous system. The antennae of moths carry thousands of olfactory structures, sensilla, which contain the olfactory receptor neurons. Within these sensilla, odour recognition relies on the expression of a diversity of olfactory genes located on the cell membranes of olfactory receptor neurons (Legeai *et al.* 2001).

There are many unresolved questions regarding the evolutionary transitions between generalist and specialist life styles among herbivorous insects. As outlined above, generalist herbivorous insects face many challenges, including the need for both physiological and behavioural adaptations to a broad range of host plants with widely differing chemical defences and odour profiles. One aspect of this question is whether generalist species, especially with extensive geographic distributions, truly maintain the same degree of generalism over their entire range, or

whether they are subdivided into local populations with some degree of specialization towards locally abundant host plants. There are examples of herbivorous insects that apparently maintain the same overall degree of polyphagy over large geographic ranges, in spite of great local variation in host availability (Wehling and Thompson 1997). In other cases, generalist species have been shown to consist of a range of separate cryptic species and host races (Blair et al. 2005).

In this study, I have investigated the degree of generalism in a polyphagous, geographically widespread pest, the Egyptian cotton leaf worm moth *Spodoptera littoralis*. *S. littoralis* is one of the leading Lepidoptera models used for studying the insect olfactory system (Jaquin-joly *et al.*, 2012). I have characterized differences in physiological adaptations and host plant preferences between two selected strains from Africa, one originating from Egypt, the east coast, and one from Benin, the west coast.

I have investigated whether host preference hierarchies in female oviposition choice differ between these two strains, using a series of five host plants for which females of the Egyptian strain have previously demonstrated a pronounced preference hierarchy (Thöming et al. 2013). This is simply to establish if there are any differences in generalism between the two strains. I have also studied larval diet adaptations on host plant in the two strains by comparing larval performance when feeding on three of the five host plants and comparing it with an artificial, semi-synthetic diet. This would show any adaptations between the strains to the host plants presented, where the hypothesis is that there are. Finally, I have also investigated whether the two strains displayed any differences in olfactory responses on their respective antennae to a series of host plant extracts and synthetic compounds, which would suggest an adaptive shift in olfactory sensitivity to host plant related compounds between these two strains. If there truly is a tendency towards adaptation during the larval phase, this would be noted in through analysing the olfactory responses. The hypothesis state that there will be a difference between the strains when comparing how they response to different host plants.

Understanding the degree of generalism trough answering these questions will aid in understanding the factors affecting the moth's choice of host plant and hence support any progress in developing a pest control in an integrated pest management system.

Methodology

The model species, *Spodoptera littoralis*

S. littoralis, Lepidoptera: Noctuidae, is a highly polyphagous pest widely distributed in the temperate regions and Mediterranean countries. It has a native range all over Africa, but has been introduced in Asian environments as well as southern Europe. The two populations used for this study originated from the African West coast (Benin) and from the East coast (Egypt), both countries mainly surviving on the agricultural outputs (Encyclopaedia of the nations, 2013).

S. littoralis has great economic impact as one of the most destructive lepidopteran pests within both tropical and subtropical regions. It attacks plants from 44 different families, containing at least 87 species of high economic importance (Salama *et al.*, 1970). The larvae of *S. littoralis* feed greedily on almost all plant organs. The young leaves are preferred, but when they have been consumed, other parts, such as stems, buds or pods are attacked too. An infestation generally sooner or later leads to complete defoliation of a plant (Bayer crop science, 2012).
In Africa it is mainly damaging vegetables, in Egypt more specifically it is known for primarily damaging cotton and in southern Europe, foremost Italy, it attacks both flowers produced in greenhouses and fodder crops (Lopez-Vaamonde, 2006).

The female is able to mate approximately 2 days after hatching, thus lays her eggs after 2-5 days after emergence, usually on the lower leaf surface of a plant. Fecundity is strongly affected by both temperature and humidity and a female lays between 1000-2000 spherical eggs in batches of 300-500. For protection the eggs are covered in scales and hair from the female's lower abdomen (Hosny *et al.*, 1986; Eppo fact sheet, 1981).
In a subtropical environment of 25°C, the eggs hatch in about four days and the larvae then pass through their six instars in roughly another 15-23 days depending on temperature stability.
Since it is the larval stage causing the most damage, control is concentrated on egg or larval stages. The density of the egg masses tolerated to avoid crossing any economic threshold significantly varies with natural predator density as well as temperature range during the lifecycle from year to year (Salama & Shoukry, 1972; Hosny *et al.*, 1986).

After pupation it takes an additional 11-13 days for *S. littoralis* to fully develop and emerge, entering the adult stage which usually lasts for 4-10 days depending on temperature and humidity (Baker & Miller, 1974). This makes a complete lifecycle about 5 weeks long.

The adult moth's dispersal ability is approximately 1-1, 5 km overnight and it can thus oviposit within a great range during its adulthood. This aspect makes it equally important to include studies of behaviour, dispersal patterns, flight range and abundance to minimize spread and to determine threshold values in the adult stage as the larval (Salama & Shoukry, 1972).
Through analysing the adult moth's perception of odours a strategy for disrupting the behaviour of finding a suitable host plants or even locating a mate can be explored.
The moth is nocturnal and has a highly developed olfactory system. The *S. littoralis* antenna comprises >60 segments and there have been a total of 6 distinct types of olfactory sensilla identified. It has been suggested that the short tricord sensilla are the most common on female antennas and used to identify non-host volatiles produced from plants not appropriate as a larval food source. The second most common, the basiconic, on the other hand seem to solely respond to plant-related compounds, developed to assist the female in finding a suitable oviposition site beneficial for her offspring (Binyameen *et al.* 2012).
The studying and understanding of something as crucial as their recognition of volatiles greatly aids in the development of olfactory-based strategies to disrupt any of these key behaviours.

Host plants and artificial diet

During the study development and preferences of the larval stage was observed. Alongside the plant based diets an artificial diet was made as a neutral alternative (see table 1). This optimized mixture contained all nutrients necessary for a fast development, while making the results more reliable through guaranteeing no previous host plant diet would influence the oviposition site during the oviposition experiments.

The recipe used to make sure all nutrients necessary would be contained, was the following:

Table 1. The constituents of the artificial diet used for feeding experiments, and for rearing moths used for electroantennographic recordings and oviposition experiments.

Artificial recipe		
Ingredient	ml	g
Water	5275	
Corn Meal (Potenta)		385
Agar (Plant Agar)		20
Malt		110
Yeast		91.25
Soy Meal		52.7
Sugar Syrup	405	
Propionic acid	25	

In Egypt the climate is subtropical to tropical and generally very dry. There are date palms and citrus groves growing naturally, but no forests even though cypress and eucalyptus has been successfully introduced. Papyrus is common as it is in most subtropical climates.
The climate of Benin on the other hand is more typically equatorial, very hot and humid and with some small forests still surviving. The climate is beneficial for coconut plantations, giving way for oil and ronier palms to grow freely along the coastal line (Encyclopedia of the nations).

During this essay it is vital to understand that the natural host plant range of the adults, in other words all plant species naturally oviposited on by each strain, is dependent on the habitat preference of the adults. That is, the adult females could also oviposit on a number of plant species which are never encountered by them in nature.
Likewise, all plant species on which the larvae feed on in nature are included in the natural host plant range of the larvae. However, the larvae may also be potentially able to feed on a number of plant species which are never encountered in nature without consequence.

For the plant based diets cotton, *Gossypium hirsutum,* cowpea, *Vigna unguiculata,* and Egyptian clover, *Trifolium alexandrinum,* were used.

The cotton plant, *G. hirsutum*, is known to suffer great economic losses in Egypt due to *S. littoralis* (El-Wakeil, 2010). It is primarily grown for its vegetable seed fiber as well as for the raw-material for textile products (NewCROP, 2012). This particular species of cotton is also considered the most important in the cotton-yielding plants and stands for the bulk of the world's cotton supplies. The plant has been introduced all over the globe after centuries of migration, something which has made it difficult to determine its origins although it has been suggested to have been domesticated in Mexico (Wendel *et al.,* 1992). Because of its present extensive cultivation in Africa and the economic impact caused by the pest, *G. hirsutum* was selected as one of the plants for the diet experiments.

In the study *V. unguiculata* was used as a diet to observe development since is very common in West Africa, where it has both the highest genetic diversity as well as the most primitive forms suggesting this area would be where it originated from. It is considered one of the most important legumes of the tropical world, being used for vegetables, grain, fresh cuts and for hay or silage (Tropical forages). Thus using it to investigate the *S. littoralis* plasticity and preference

towards *V. unguiculata* an interesting complement to cotton, especially since it has previously been compared and considered a suitable host of *S. littoralis* (Thöming *et al.*, 2012).

The *T. alexandrinum* in Egypt is known to have large pest problems and is mainly considered a legume used in winter fodder crop fit for livestock since it is rich in proteins (Khan *et al.,* 2002). Since domestication site is suspected to, after examining the close relationship between its successions, originate from Egypt and Syria, another interesting observation could be that these native species ought to have co-evolved and adapted quite well (Badr *et al.*, 2008). This made it an obvious choice for diet experiments and an interesting complement to the oviposition experiments.

All plants were grown in a climate controlled biotron before used as diets in the experiments. They were kept in a stabile temperature of 22 °C, a relative humidity of 70 % and a day length of 16 hours (from 04.00 to 20.00).

Physiological adaptations of larvae to host plants

Differences between the two strains (Egypt and Benin) in their ability to digest and utilize a spectrum of host plants as larval food were evaluated by comparing their performance on four different substrates: cotton, cowpea, clover and artificial diet.

The experiment was initiated with 2-3 day-old *S. littoralis* larvae originating from Egypt and Benin, respectively. Each population was divided into 4 boxes, 25 individuals in each, which were fed cotton, cowpea, clover or artificial diet. This experiment was repeated twice with two independent groups of larvae for each diet, for a total of 50 larvae tested per diet. They were kept in a controlled climate chamber with the temperature of 25+ °C, a relative humidity of 70%, 17 hours of day and 7 hours of night. The larval performance was measured as four fitness-related traits: survival to pupal and adult stages, growth rate, and pupal weight. During maturation the larvae were weighed every 3: rd day from day 10 except for larvae on artificial diet, which were weighed from day 7 due to the fast maturation of larvae in this group. During this time it was noted how many survived out of 50 on each diet to measure the survival rate. When individuals reached the pupal stage they were sexed, separated into boxes according to pupation date and kept in the climate chamber. The weight of the pupae was noted within 24 hours. Once the new adults emerged, the number of days from pupation to emergence was noted.

Adult oviposition choices

To investigate the variation in oviposition preferences between the two strains of *S. littoralis*, a five-choice test comprising a selection of plants from North and West African agroecosystems was executed.

The adults used for this were raised on an artificial diet, unaffected by any previous experience related to the plants tested. The two populations, Egypt and Benin, were observed separately. The choice of oviposition site they made was between maize, cowpea, cotton, clover and cabbage, five plant species from four different families and a part of the natural flora. All plants were grown for approximately 3 weeks before used in the diet experiments. Climate was controlled in a biotron, using a day length of 16 hours (from 04.00 to 20.00) and a stabile temperature of 22 °C. Before use, all plants were controlled to not carry any external damage which could affect the oviposition choice. The plant material was all used in a vegetative stage, not flowering and taller plant species were used while still less than 1 meter in height.

Oviposition experiments were performed in 2*1*1 m cages, made from wooden frames covered with nets with a mesh size of 3 mm. Tests were conducted using groups of 5 cages in parallel, with the positions of the target plants always varied in five different configurations according to figure 1 in order to minimize position effects.

Adult males and females of *S. littoralis* were mixed in a mating box to ensure mating. After the mating commenced, a single couple was moved to each cage. The cage was then left for 3 days to ensure sufficient time for oviposition to take place, with checking up every day to make sure of progress. After 3 days the last egg batches were collected before cleaning of the cages including freezing or quarantine to ensure that no additional larvae survived. In each cage the combined weights of egg batches on each plant were noted and used as a replicate.

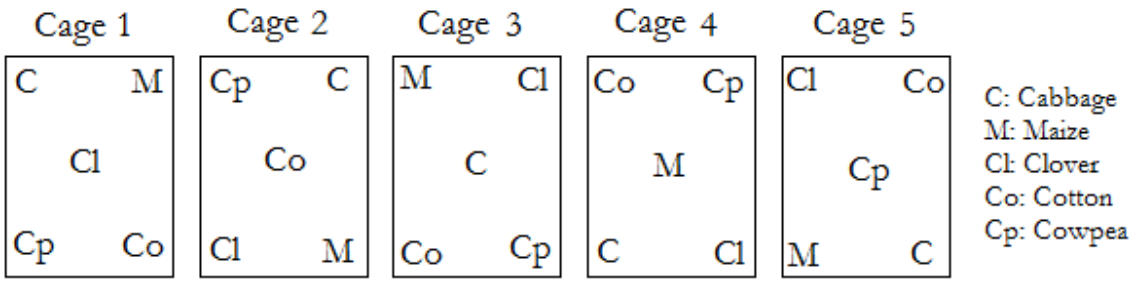

Figure 1. The five cages set up used each 5-choice experiment. The order was changed to avoid any positional effects.

Plant odour headspace collection

To collect chemical compounds emitted by host plants for subsequent EAD studies and selection of synthetic test compounds, headspace collections was conducted. Time constraints did not permit collection and chemical characterization from all test plant species used in behavioural experiments. Instead the plants used were clover; the plant most protecting against predators, cowpea; a host plant of the Benin population and cotton; the natural occurring host plant for the Egypt population. Also a control was done with no plant, only air passing through.

The different headspace collections were performed repeatedly, with collection from all plants done in parallel to ensure similar conditions. Pots with plants were enclosed in separate polyacetate oven bags and left for 10 days, in 12 hour intervals. Air was pumped through at a rate of 150 ml/ minute in filters filled with 100 mg of Porapak Q, 50 – 80.
Before use they were rinsed with pentane (2 * 500 µl) and hexane (6* 500 µl). To rinse the filters after collecting, each filter was run through with 600 µl of hexane in hope to elute 500 µl of solution from the Porapak.

The concentration after rinsing the filters was 1, 44 minutes/ µl, that is for each µl solution produced, the filters has been collecting volatiles for 1, 44 minutes. For the EAD a higher concentration was used by pooling the 10 samples together and concentrating them with nitrogen gas. After the concentration, the solution used was at 14, 4 minutes/ µl.

Identification of odour constituents: GC-MS

Compounds from the headspace solutions used for the EAD was identified through Gas chromatography–mass spectrometry (GC-MS) coupled. A 2 µl extract was used from each of the collections and injected to the GC. Carrier gas was helium and the same temperature program as for the EAD, it started at 30 °C and went up to 230°C increasing 8°C every minute and equipped with an HP5, non-polar column.

This was done before the pooling to confirm the solutions purity as well as after the concentration to better confirm the FID peaks. The FID peaks present was identified through comparison with the NIST-, Alnarp- and Wiley library.

The EAG responses to individual compounds were quantified as the amplitude of the deflection from the baseline (in mV). In order to minimize variation in absolute responses between antennae, each response was normalized to the response to the main pheromone component,

which constituted a standard reference stimulus that was puffed every third time. The normalized response to individual stimuli was thus quantified as the proportional response compared to the average responses of the two reference stimuli puffed before and after the test stimuli. Comparing them between the strains hence showed any differences in relative sensitivity towards a specific compound.

GC-EAD and EAG techniques were used to confirm any differences in relative antennal sensitivity between the different strains to compounds emitted from suitable host plants, which would suggest that adaptations in olfactory sensitivity may have occurred based on the local odour environments.

GC-EAD and EAG

These techniques have a history of successfully identifying several behaviour modifying compounds from a wide range of insect semiochemicals, mainly sex pheromones, but also aggregation and kairomones in numerous pest species (Bruce, 2000).
GC-EAD (Gas Chromatography-Electroantennographic Detection) is thus one of the most important techniques in seperating pheromones and other semiochemicals of insect compounds is the use of the gas chromatograph (GC) joined with an electroantennographic detector (EAD, electrophysiological recordings of an insect antenna) to confirm these cues (Byers, 2004; Larsson & Svensson 2005). The technique is mainly useful for identifying compounds rather than the differences in responses.

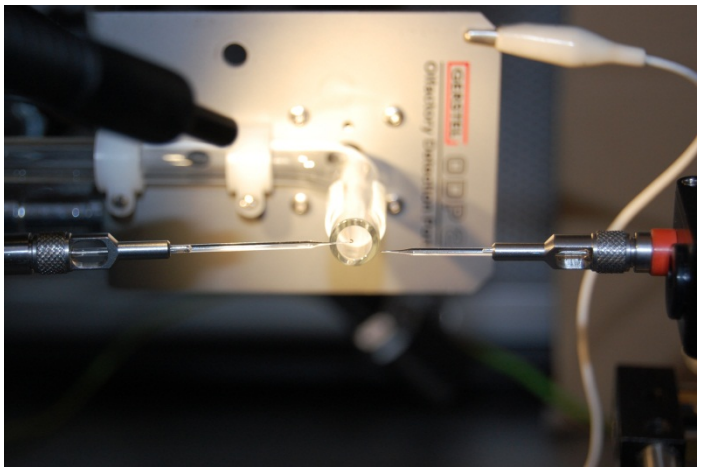

Figure 2. The set up for an EAD. Between the glass capillaries an antenna is mounted and through the pipe the odours pass through as the GC's temperature rises.

The gas chromatography is used for separating any components in an extract by vaporizing it and detecting how quickly they travel through a column containing an absorbent material

(National Academy of Sciences, 2003). By coupling the two techniques, they are used in order to separate components and determine any antennal activity towards each separate component of the extract.

An extract is injected into the column and separated by the increasing temperature, half the material is sent to the flame ionization detector (FID) and the other to the antennal mounting. The FID and antenna are recording simultaneously allowing the peaks from the GC and the responses from the antenna to be observed in parallel (Figures 2, 3; Larsson & Svensson, 2005).

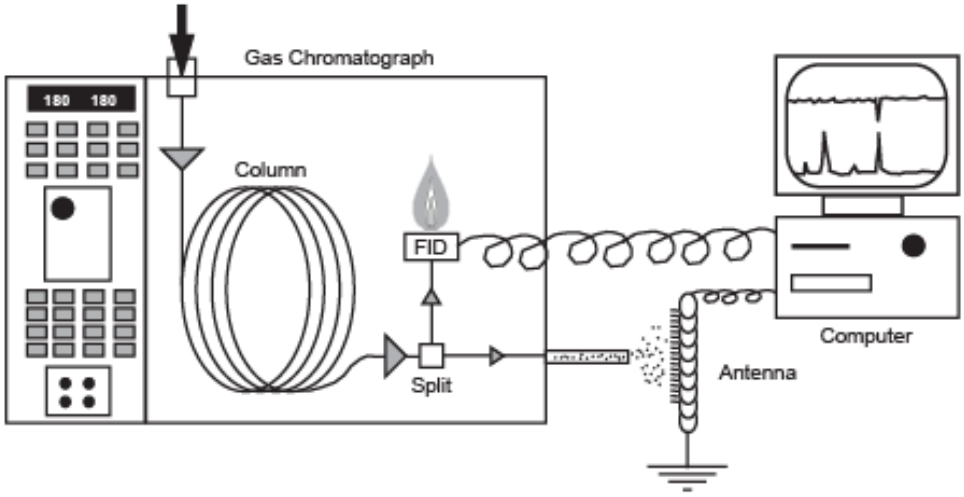

Figure 3. The technique used for Gas Chromatography-coupled Electroantennographic detection of physiologically active volatiles in biological extracts (GC-EAD). The sample is injected into the GC and separated into its components on a capillary column. At the end of the column, the amount of each compound eluting is split into two equal fractions, which are simultaneously detected by the Flame Ionization Detector (FID) and flushed over the antenna. Any responses from the antenna are registered in parallel with the signal from the detector. From Larsson MC & GP Svensson (2005), Methods in insect sensory biology.

The solutions used in the EAD were all collected through headspace and concentrated. The plants used were the same as for the diet experiments; cowpea, clover and cotton.

For every reading a new antenna are excised, mounted between two glass capillaries filled with ringer solution and placed between two silver electrodes. From this an amplified signal was recorded by the computer software GC-EAD 2011, V.1.2.3, Syntech.

A 2µl sample of the solution of interest was injected into the GC. After GC separation, any chemical signal that matched to an EAD peak signal indicated the existence of a potential semiochemical (Byers, 2004). An HP5, non-polar column was used in the GC. The temperature program used started at 30 °C and went up to 230°C increasing 8°C every minute. The carrier gas used was hydrogen.

The EAG technique was used as a complementary method to GC-EAD. In the EAG recordings stimuli were presented as a pre-selected set of individual synthetic test stimuli, delivered via

odour cartridges to the antenna. In each odour cartridge, 10 µL of a solvent (hexane or paraffin oil) with known concentrations of the compound, were pipetted onto a filter paper (0.5 x 1.5 cm) inside a Pasteur pipette. The Pasteur pipette was capped at the back end with a 1 mL blue Finnpipette tip. Odour stimuli were delivered into an air stream flushing over the antenna by puffing 2.5 mL of air through the pipette, controlled via a stimulus device (Syntech). This allows only for a more limited selection of stimuli than the total number of compounds present in the headspace extracts, but provides a more robust method including more standardized comparisons between individual antennae.

For every EAD and EAG a new virgin female was used. Cutting of the first antenna at the base and mounting it between the capillaries of the EAD only one session was completed. After that, excising the second antenna an EAG recording was conducted with a selection of synthetic compounds. All the headspace samples (clover, cotton, cowpea), as well as EAG recordings, were performed 5 times on individual antennae from each population. For the EAG, a series of compounds identified from the headspace samples as well as from the literature, considered physiologically active on the *S. littoralis* antenna, was used. All compounds were diluted in paraffin oil (1 µg/µl), except Nonanal, β-myrcene and β-caryophyllene, which needed 10 µg/µl to give any response. They were arranged in order of the slightest to the greatest response and puffed in the order shown in Table 2. The main pheromone component, which was used as a reference stimulus (see below), was diluted in hexane.

Table 2. Listing of all the compounds used as stimuli for the EAG in order of stimulation. Solvents: H = Hexane, P.o. = Paraffin oil.

Stimulus compound	Solvent	Dose	CAS number	Source
Paraffin			8012-95-1	Chemicalbook
Main pheromone component((Z)9,(E)11-14:Oac)	H	1 µg/µl	50767-79-8	Chemicalbook
(E)2-hexenal	P.o	1 µg/µl	6728-26-3	Aldrich
(E/Z)-b-ocimene		1 µg/µl	3338-55-4	PubChem
(Z)-3-hexenyl acetate	P.o	1 µg/µl	3681-71-8	Aldrich
α-pinene	P.o	1 µg/µl	7785-26-4	Aldrich
Nonanal	P.o	10 µg/µl	124-19-6	Aldrich
(-)-linalool	P.o	1 µg/µl	97 78-70-6	Aldrich
β-myrcene	P.o	10 µg/µl	84776-26-1	Chemindustry
β-caryophyllene	P.o	10 µg/µl	87-44-5	Aldrich

The quantification of compounds was made through normalisation of the pheromone puffed every second compound. By comparing the millivolt each response of a compound gave with the normalisation of the two pheromone responses average a percentage was given. Comparing them between the strains hence showed any differences in sensitivity towards a specific compound.

Results

The 5-choice host plant experiments of the Egyptian strain demonstrated a preference towards laying eggs on clover and a complete avoidance of maize (Figure 4). The N value of the 5-choice experiment with Egyptian *S. littoralis* amounted to 20 replicates.

In the 5-choice experiments using the Benin strain, clover also seemed to be the more popular choice, whereas they displayed a clear avoidance of cowpea. The N value was a total of 14 for the Benin strain during these experiments.

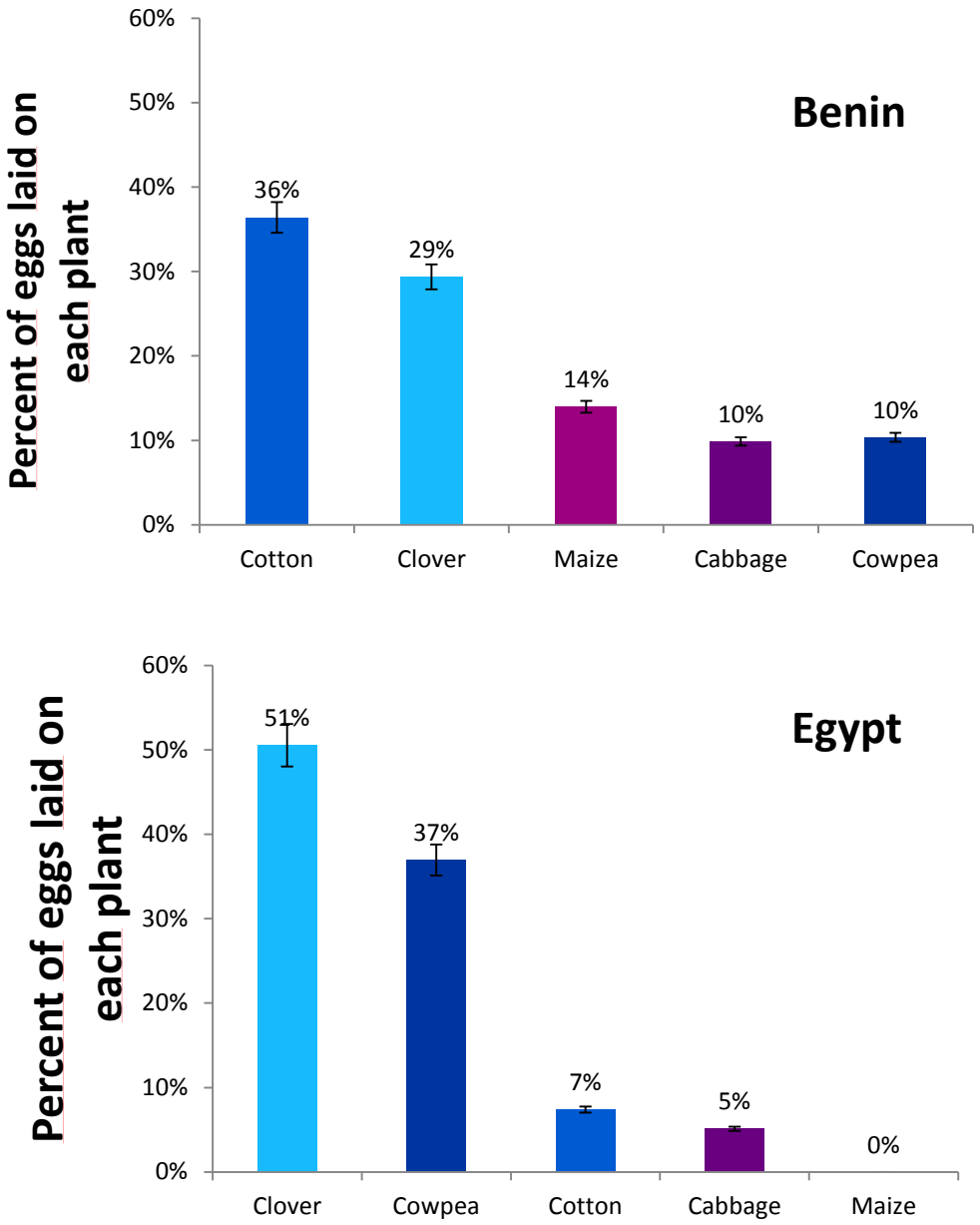

Figure 4. The oviposition preferences of the Egyptian and Benin strains of *S. littoralis* during a 5-choice test. The charts show the average percent of total eggs laid on respective plant (± S.E.). When comparing the two strains a percentage was calculated through egg batch weight divided by total weight of all batches in the same cage and an average was calculated from this data.

The Egypt strain completely avoided to oviposit on maize, which can be seen in figure 4. The Benin strain on the other hand shows a preference towards cotton, followed by clover, maize and in the end cabbage and cowpea. Significance can be seen in preference towards clover after a T2 test (appendix 1). Further analysis with a Multivariate Anova (appendix 6) gave a P-value of 0,09; showing a near significant difference between the overall oviposition patterns on the host plants of the two strains.

During the diet experiment done using the Egyptian population and disregarding the non-natural artificial diet, clover proved to have a notable effect on the larval weight gain, followed by cowpea and cotton.

Comparing the significance between the diet curves within the Egypt strain, based on the data seen in figure 5, show a value implying a difference in development on the diets between artificial (a), clover/cowpea (b) and cotton (c). Within the Benin strain there can be seen a significance between all diets (see appendix 4). Also, between the two strains significance in development based on diet amongst cowpea and cotton is shown, where the Egypt strain develops better on cowpea and the Benin strain better on cotton (appendix 4).

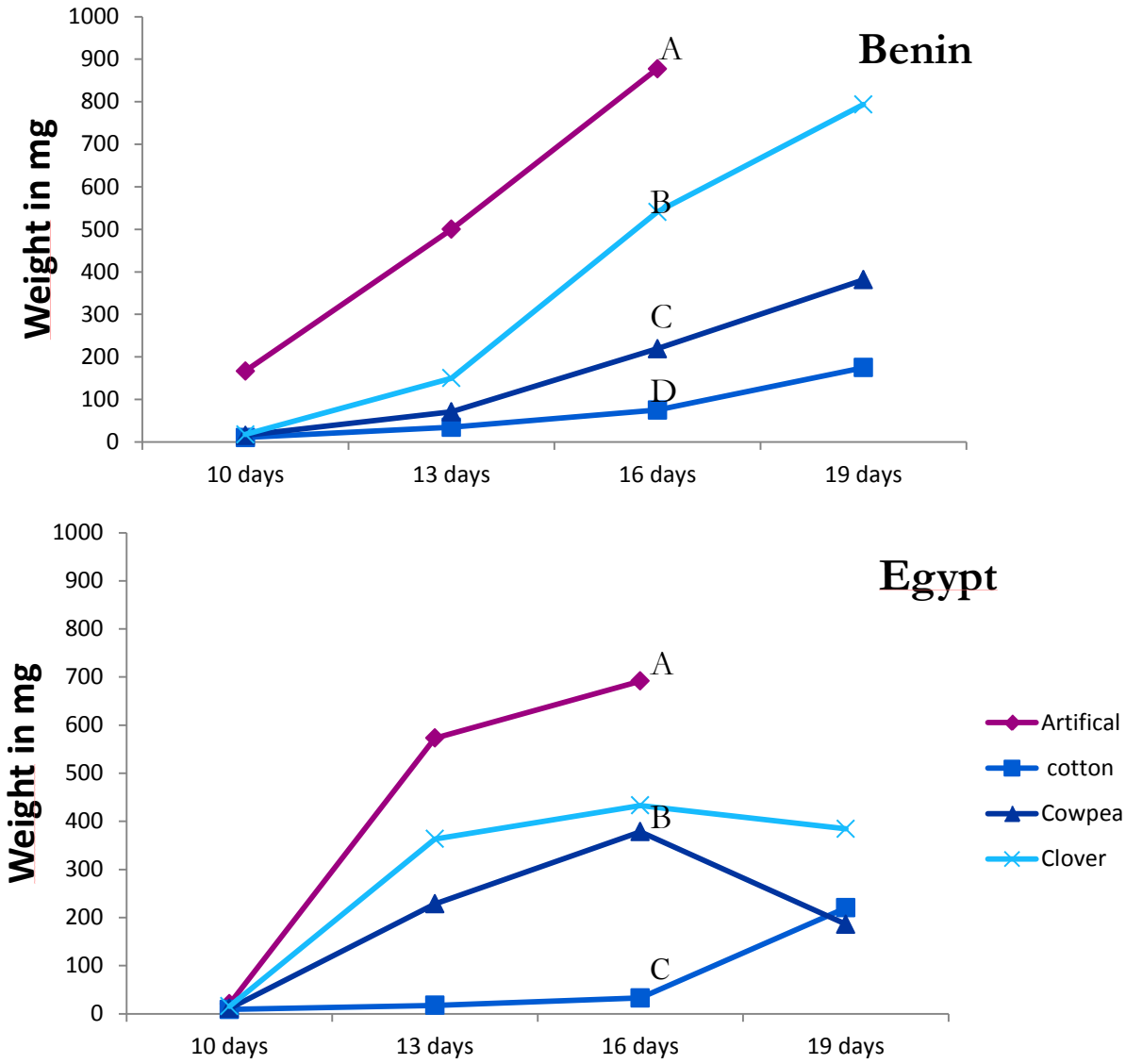

Figure 5. Average weight curves of the Egyptian and Benin strain respectively from the diet experiments. Both cases are showing the most efficient weight gain can be made on clover, cowpea and cotton respectively, disregarding the artificial diet. To calculate the significance between the weight within a strain after 16 days a Tukey's test was preformed which showed significance between all diets in the Benin strain and between artificial, clover/cowpea and cotton respectively. Between the strains significance was calculated with a T2-test, illustrating that the Egypt strain develops better on cowpea (0,000) and the Benin strain on cotton diet (0,001) after 16 days.

The survival rate of each strain showed no significance on different diets during the 19 days.

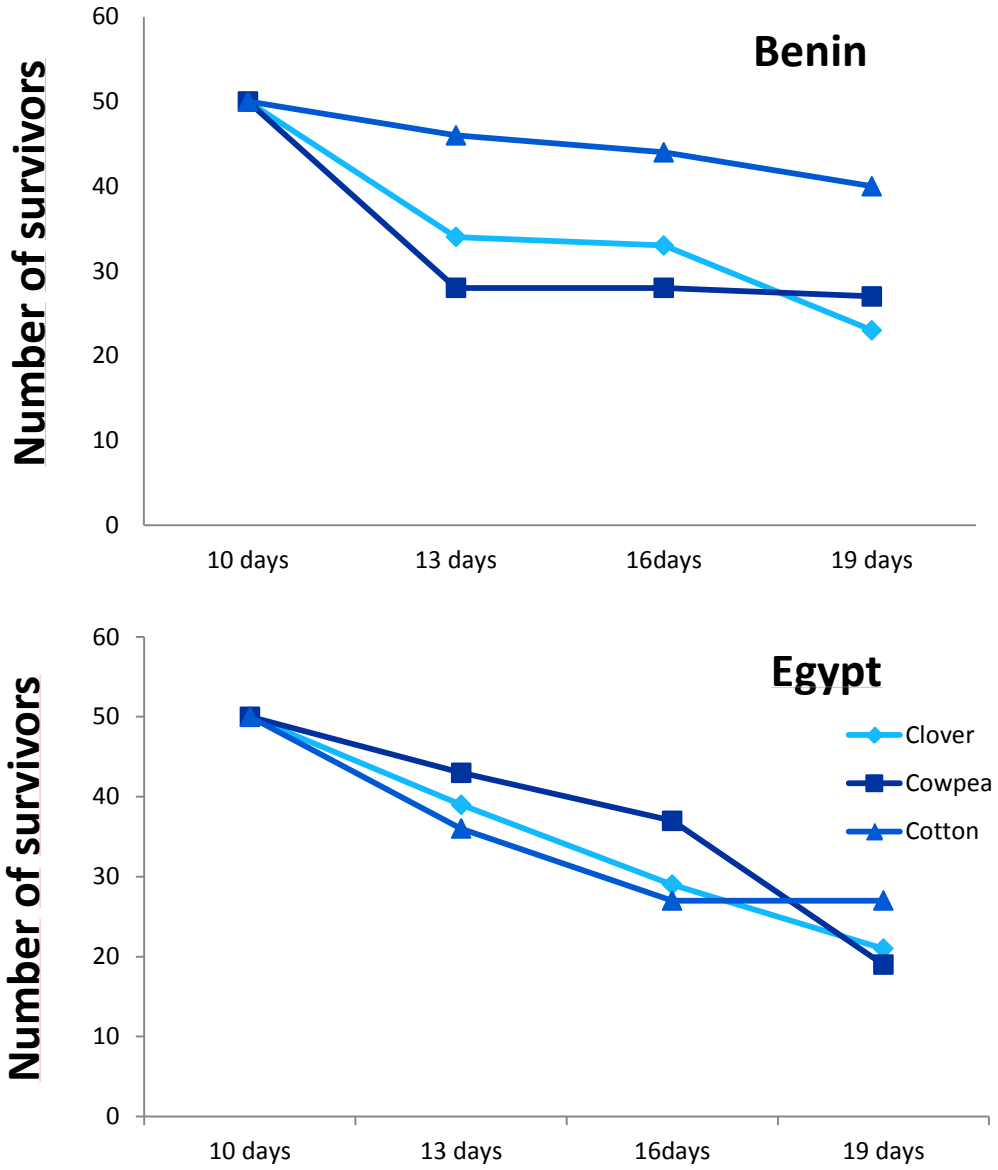

Figure 6. The survival rate of both Benin and Egypt strain of *S. littoralis* on the different diets; clover, cowpea and cotton. Two groups with 25 individuals in each category were conducted separately. When conducting a t2-test no significance was shown, suggesting the diet during the larval stage does not affect survival.

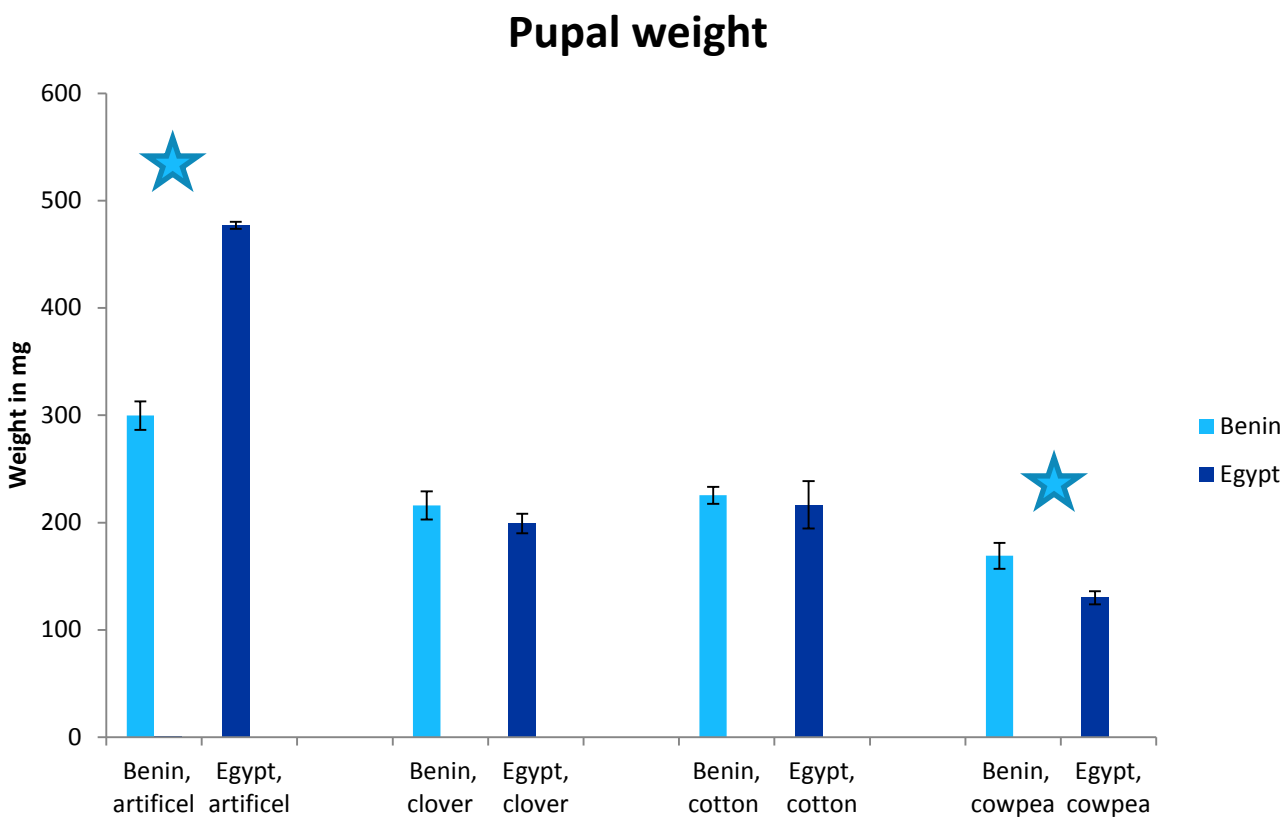

Figure 7. Comparison of pupae weight (averages ± S.E.) after the diet experiment between the two strains of *S. littoralis* on all the diets; artificial, clover, cotton and cowpea. To establish if there were any significance between the two strains depending on diet a T2-test was conducted for every diet.

The result from figure 7, the pupal weight diagram, mainly show a significant difference between the two strains on cowpea and artificial diet, where the p-value amounted to 0,008 and 0,002 respectively (see appendix 1).

During the recording of EAD several responses were found between the two strains. Figure 8 through 10 illustrates the responses to the different plant extracts. The few responses not corresponding to a peak has not been accounted for in these graphs.

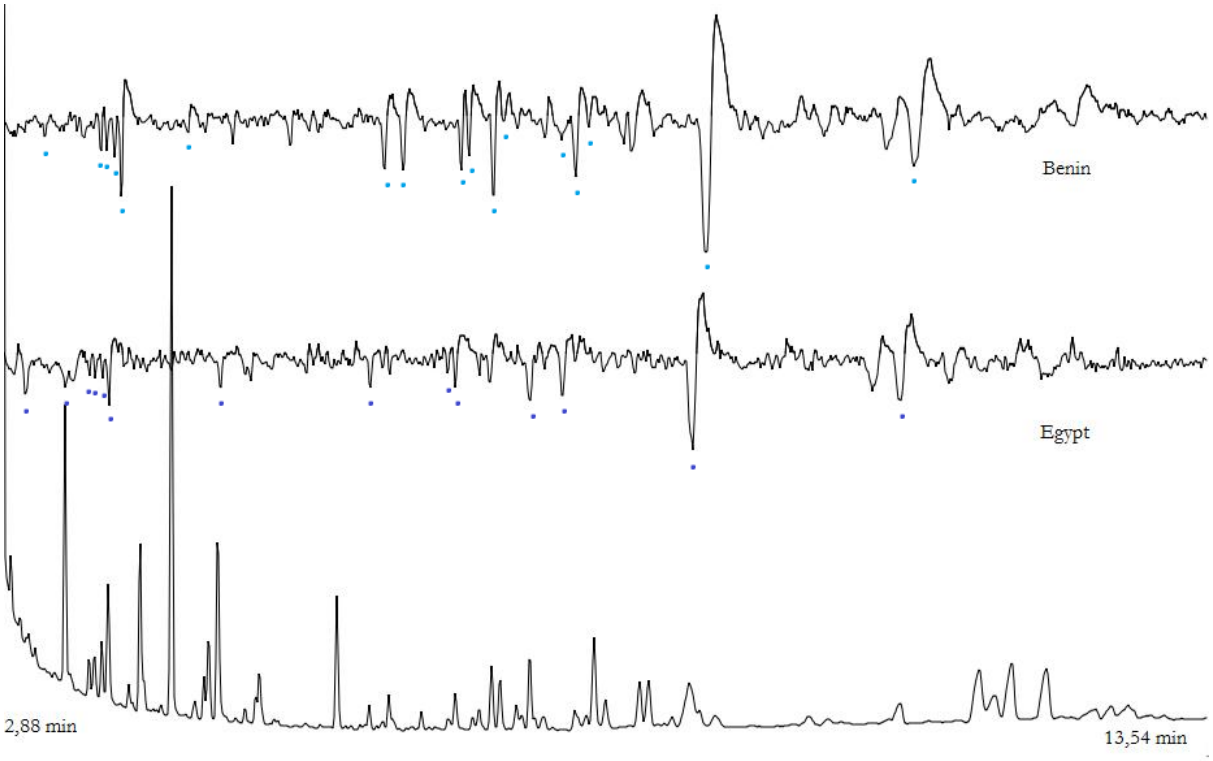

Figure 8. EAD recorded of *S. littoralis* exposed to a cotton extract. Responses occurring repeatedly throughout the recordings and corresponding to a peak on the FID have been marked with a dot.

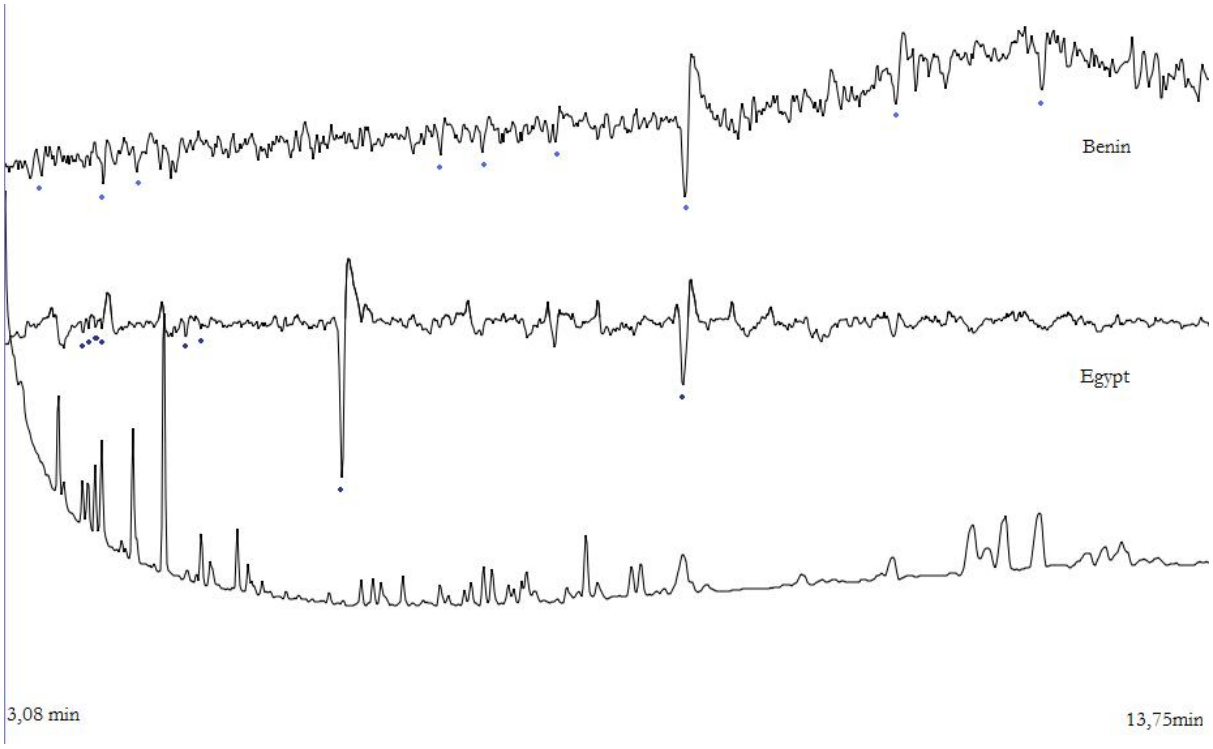

Figure 9. The EAD of the two *S. littoralis* strains when exposed to clover extract. Responses occurring repeatedly throughout the recordings and corresponding to a peak on the FID have been marked with a dot.

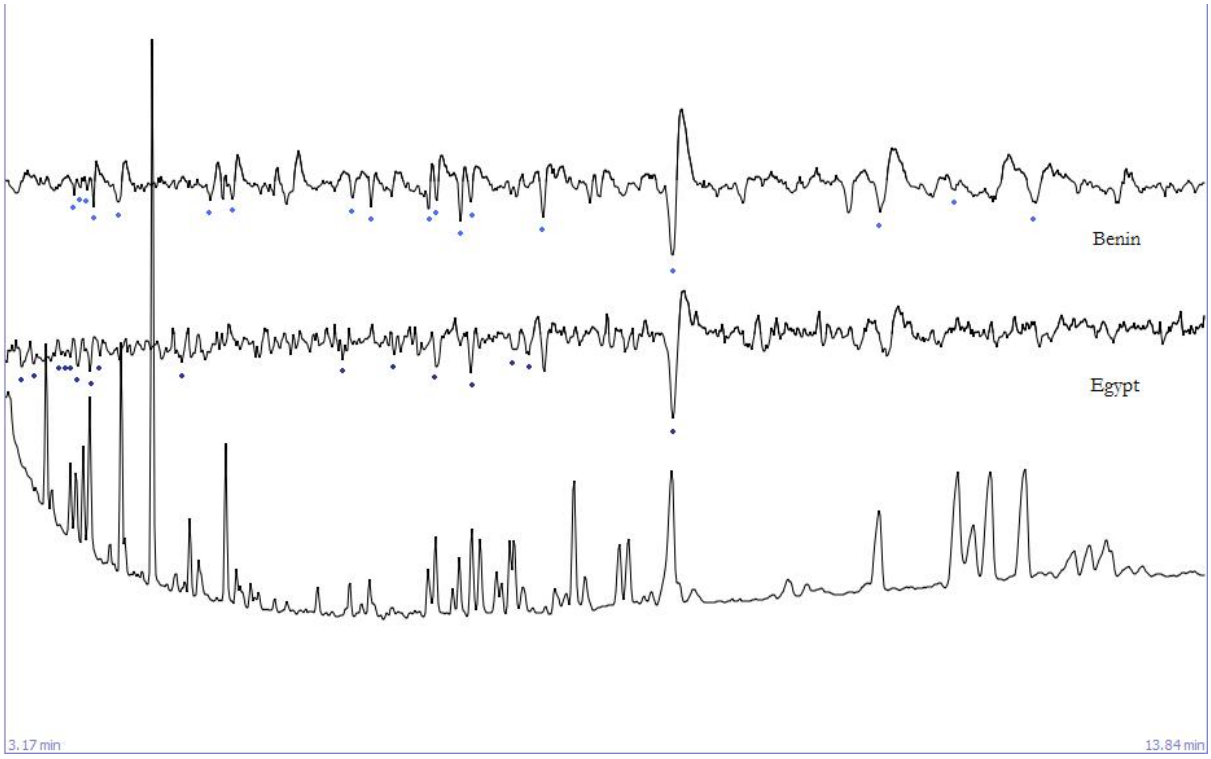

Figure 10. The EAD recording of exposing cowpea extract to both strains, Benin and Egypt of *S. littoralis*. Responses occurring repeatedly throughout the recordings and corresponding to a peak on the FID have been marked with a dot.

Due to variations between individual antennae appears to be greater than between strains, it is difficult to compare the physiology based on the results from the GC-EAD. Instead a comparison between the EAG was made.

The data summarized in figure 11 shows that the Benin strain displays a tendency for less sensitivity towards every compound used during the EAG. Significant values found between the two strains through T2-tests can be seen in appendix 5 and shows in every sample except α-pinene and β-caryophyllene, a higher sensitivity for most compounds used in the Egypt strain. Figure 12 show an EAG recording where the depolarisation was measured in millivolt.

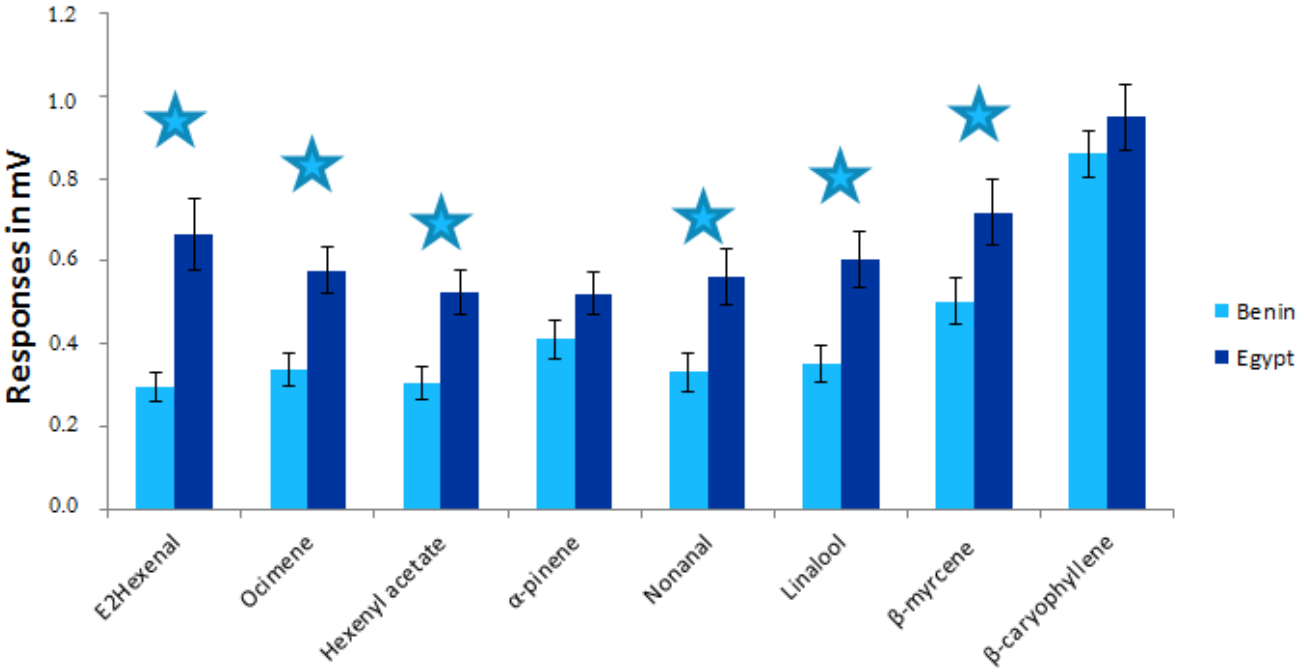

Figure 11. Comparing the quantificated responses between the two strains of *S. littoralis* from Egypt and Benin from the EAG. Comparing the responses from both strains was made through an average of all responses in millivolt. To vindicate the significance between the responses a T2-test was conducted by comparing the mV from each compound response. Shown in the picture is the average of mV used for the T2 test.

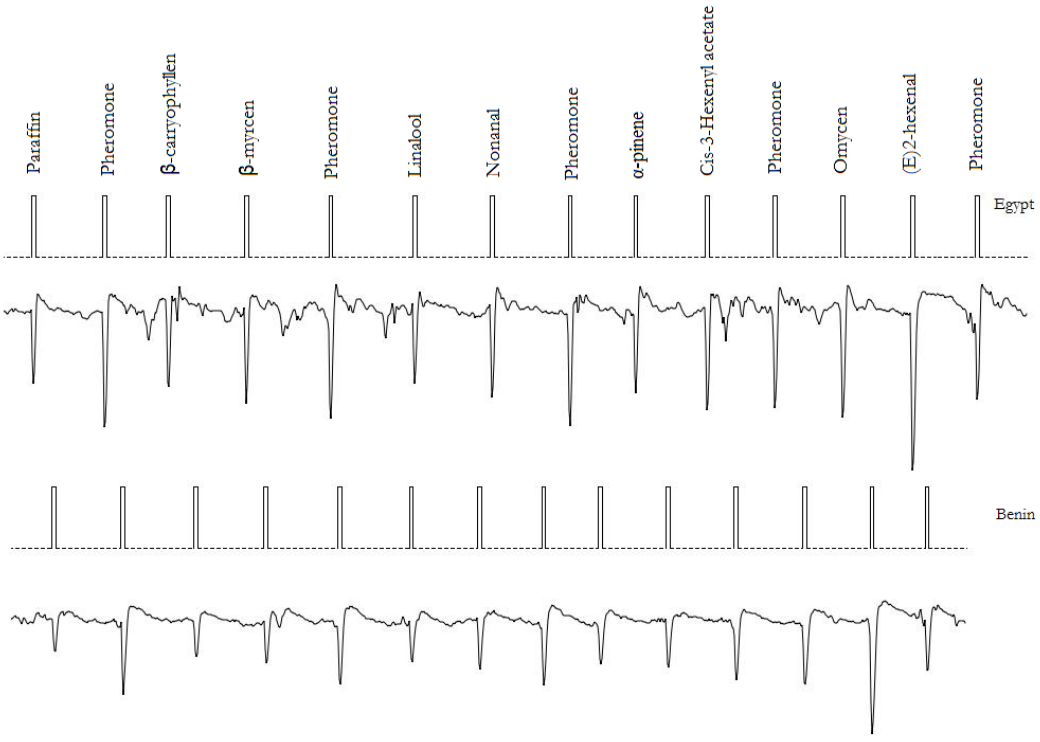

Figure 12. A EAG recording showing the responses to respective compound used to quantify the sensitivity.

Discussion

Survival and adaptation

The relationship between insects and plants is considered to be maintained by two separate processes;
1) The adaptations of insects to a specific host plant, suggesting a co-evolutionary relationship between specific species of insects and plants.
2) The correlation between adult oviposition preferences and larval feeding preferences, thus the co-evolution between the hosts plants range of adults and larvae (Wiklund, 1975).
Adaptations of insects to specific host plant species are determined by a number of factors, such as geographical and habitat overlapping of insects and plants, physiological suitability of various plants as food, abundance of those plants, as well as the amount of predation, parasitism, intra- and interspecific competition occurring.

All results from both the development and the survival experiments, figure 5 and 6, showed the same similar hierarchies between the host plants, regardless of diet or survival. This suggests that there is a similarity in the natural fauna and the ability to digest these plants, as well as how any further adaptations to the local habitat have been copious. It also displays how closely the two strains are related.

The complete shifting and adaptation from one host plant to another by the larvae, preferably to one without any damages or growth inhibiting chemicals is extremely time-consuming. A larva of *S. littoralis* which has already adapted to a specific host, tend to choose this plant over others. This is reasonable since the insect adaptation to a specific host, involves genes adjusting and expressing a series of specific detoxification enzymes and even salivary proteins to compensate for those plants specific defences (Snyder & Glendinning, 1996).

In this way, the larvae accustom itself on a sensory level. This also helps overcoming any disliking due to compounds found in the diet of choice (Glendinning *et al.*, 2009).

This custom taste is mainly learnt already in an early stage of development and carried on through metamorphosis and affecting the choice of oviposition site and becoming advantageous for the offspring during early instars while the food source is fixed (Insect-plant biology).

A deliberate metabolic modification and host plant change has its benefits though, such as development time decreasing to better adjust to climate or avoiding seasonal predators. An alteration in taste is usually complex, but habituation and alteration can start already within 12-24 hours after a diet change (Glendinning *et al.* 2001). In other words, this strong polyphagous behaviour and capability of adaptation, allows the larvae to change host or even find a more suitable one within a day.

In the case of *S. littoralis*, the sensitivity of chemoreceptors increases for host-specific compounds when a larva is reared on a specific plant. For example, when reared on cabbage *S. littoralis* have shown a much higher sensitivity towards glucosinolates then reared on an artificial diet lacking such compounds. Similar differences have been observed in the uptake pattern of protein and carbohydrate. Larvae reared on a diet low in carbohydrates decreases the sugar level in the heamolymph, which in turn decreases the sensitivity threshold of the receptors for sugar (Simmonds *et al.* 1992).

During the experiments made, only one diet was made available throughout the life cycle. Having the earlier generation feed on an artificial diet, thus not having any recent preferences affecting the choice, this provides a more detailed view of how a specific diet affects development. This offers an idea of on which crop the development time would be the least, further showing on which crop the pest would be more severe, bringing more generations during one season. Also, it gives information on how this development time needs to be taken into consideration when applying any pest control and how long before you need to apply it before reaching the threshold limit.
Even more so, having the results show a greater body weight means fewer survivors shows that even more factors needs to be considered before using any pest control.

As opposed to these experiments, the frequent switching between hosts plants in nature, without adapting, have shown to increase the survival rate of many other insects. The two suggestions of physiological advantages when feeding on mixed diets could be that it results in a better uptake of optimal compounds and nutrient compositions as well as preventing or at least dilute the uptake of toxic secondary metabolites from some of the host-plant species (Insect-plant biology).

This phenomenon of preference induction shows that insects not only choose between suitable host- and non-host plants, but also between plants equally acceptable as host plants and perceive a very detailed odour profile for each plant species considered. It also shows the complexity and

precision involved in host recognition, based on both taste and olfaction sensory (De Boer & Hanson 1984).

Weight

Moth males choose and compete for the fittest females by responding to their odour, based on their ability to synthesize a more attractive sex pheromone blend. It has been suggested that females producing the favoured blend of sex pheromone are generally heavier than average which is associated with a larger stock of attractive compounds (Jaffe *et al.,* 2007).
There are several factors affecting something as variable as adult body weight, most vitally the availability and quality of larval diet (Torres-Vila *et al.*, 2005)
This suggests that the adults would choose the host plant giving a diet most suitable for their offspring's weight gain to ensure future reproduction. Conducting the diet experiment and comparing them to the oviposition choices of each strain will therefore reveal any specific adaptations to local host plants and the nutrition they offer.
The larvae of all moth species generally feed on a more or less restricted range of plants. The way in which the host plant choice is related to both moths and plants is mainly the result of (1) the oviposition preferences of the adults, (2) the host plant preferences of the larvae or (3) a combination of both assuming the habitat allows it (Wiklund, 1975).

Observing the diet curves in figure 5, both strains seem to have the same order in diets beneficial for larval weight gain; clover, cowpea and cotton. When relating the weight from day 16, before the larvae on artificial diet went into their pupal stage, within the strains there is a significant difference on how they develop on each diet. The Benin strain significantly develops better on an artificial diet, after that clover, cowpea and cotton respectively while the Egypt strain matures best on artificial, clover/cowpea and cotton in order. Furthermore, comparing the weight between the strains show significance between the developments in cotton as well as cowpea diet. The Benin strain seems to significantly develop a greater body mass on the cotton diet compared to the Egypt strain and the Egypt strain better on the cowpea diet compared to the Benin strain (appendix 3).
The Egyptian strain shows a slightly better development on cotton instead of cowpea day 19, which can be explained thru the natural weight loss before pupation. Other than that there are no noticeable differences in weight gain due to diet between the two strains, suggesting they have similar ability to digest the same plants.

Pupal comparison

In the final weight chart, figure 7, a higher pupae weight is present in the artificial diet where the Egyptian gained considerably more body mass than the Benin strain, possibly since the Benin larvae acquired for the experiments have not been retained in a laboratorial environment for as many generations as the one from Egypt. This could very well be the result of the Egyptian *S. littoralis* adapting to use the nutrients from the artificial diet more efficiently than the Benin population since it has been kept longer in a laboratorial environment and hence been reared on it through more generations. Being able to digest the nutrients available from this mixture more efficiently this results in the greater pupae weight for that strain.
The figure also shows significance between the strains fed on the cowpea diet, where the Benin strain has a higher weight and thus has developed better. This correlates with the weight curve in figure 5 where after 19 days the Egypt strain show a greater weight loss than the Benin strain on the cowpea diet.

Oviposition behaviour

Decision making

Herbivorous insect species indicate that there are limitations on efficiency of the decision-making process. This involve time spent making a decision, ability to make the best choice among hosts of variable quality and levels of distraction during a host-related activity. It is likely that compromising between both host quality and time taken for the decision is difficult. The individual may make very good decisions in accordance to host quality, but take time to make them, or the individual take hurried decisions, perhaps on the behalf of host quality (Bernays, 2001).

Earlier studies including four species of generalist larva of Lepidoptera, showed that when a choice was offered, individuals generally did not select the most favourable host to oviposit on for growth or development of the larvae.
Although there may be various explanations for this behaviour, the data are consistent with the notion that making the most appropriate choice correlates with time. One of the lepidopterans used for this study, *Grammia geneura*, showed, although decisions could be made quickly, they tended to be poor and with no respect to what was the best choices for larval development (Bernays & Minkenberg O, 1997).

It also showed that individuals were highly likely to move away from the best food plant if additional plant species were present, but they were most likely to stay on the food plant if only conspecific plants were in the area (Bernays, 1999). These data propose that the individuals were distracted when there was an additional food source present, which may have complicated their decision making (Bernays, 2001).

The 5-choice experiment conducted in this study and illustrated in figure 4 show the hierarchy in the Egypt strain: clover, cowpea, cotton, cabbage and maize and in the Benin strain: cotton, clover, maize, cabbage and cowpea. Clover ranked relatively high in both strains, ranking higher in the Egypt strain than in the Benin strain.
After leading a Manova test, it proved difficult to analyse the results correctly since the experiment set up results in a lot of zero data, i.e. plants which has no egg batch on them in each replicate was noted with a zero in weight. This makes the variation too high for 5 plants to guarantee a reliable analysis which is why analysis between each plant is a better option to see any significance.
Significance could however be seen between preferences after conducting a T2 test, suggesting that the Egypt strain would be more attracted to clover than the Benin strain.

Clover was also the better choice considering developing a greater weight during the larval stage explaining its popularity, which also coincides with earlier studies presenting that a better choice for the offspring is chosen if enough time is given.
Between the strains the Egypt strain show a stronger preference towards its favoured hosts while the Benin strains are more levelled.
In field, far more factors needs to be considered before any choice is made, affecting decision and the time possible to spend on it. The main difference from the experiments conducted and field would be competition and climate. Supposing the *S. littoralis* would be choosing this host over others even in field, integrating clover could be a divertive strategy for pest management. Using the clover to lure the moth to oviposit on instead of cotton, removing the clover continually could have a great impact and minimize damage done to the main crop.

Oviposition preference

Even though the strains oviposition hierarchy differed, both strains somewhat favour to lay their eggs on a clover plant, uninfluenced by earlier experience to any plant material. This corresponds

with choosing based on weight gain since the diet experiments show that this is also the favourable host for a higher body mass.
Gaining weight just before pupation is essential for a successful development into adulthood, though survival does not seem to be taken in consideration since no correlation can be seen between the results in figure 5 and 6. In a more natural environment, where more choices and a more varied diet is possible, it is a reasonable choice to make for young larvae to gain as much body mass as possible in an early stage of life. Since there would exists more sources of beneficial nutrients in a natural flora, more developed larvae would also be able to choose gradually freely.

It has been established that *S. littoralis* females prefer host plants on which attacks by natural enemies are less likely (Sadek *et al.* 2010). In 1987, Damman showed that protection from a host plant was dominant over nutritional factors, which might explain the attraction. The protection the plant itself offers has to be considered, whit its many leafs and thick composition making it hard to detect or capture the larvae, thus making it a more attractive choice, especially in field. This could very well support the choice of oviposit on clover.

Differences in host adaptations between larvae and adult

Comparing the Egyptian and Benin strain during the oviposition experiment illustrated in figure 4 shows that both strains have a different hierarchy. The Benin stain shows a preference towards cotton, followed by clover, maize and in the end cabbage and cowpea, while the Egypt strain prefers clover, cowpea, cotton and cabbage while completely avoiding maize.

The difficulty with comparing results so dependant as these makes any conclusion somewhat vague and unreliable. Conducting a Manova test showed the difficulties since the experiment set up results in a lot of zeros, plants which has no egg batch on then in each replicate. This makes the variation too high for 5 plants to guarantee a reliable analysis which is why analysis between each plant is a better option to see any significance. Significance can be seen after conducting a T2 test in preference towards clover where the Egypt strain seems to be more attracted to clover than the Benin strain.

When further comparing the oviposition selection with the diet experiment it shows that clover is the most beneficial plant for development and gaining weight during the larval stage, as well as the pupae weight, indicating why it's placed so high in both strains.

The reason for choosing hosts in field might be influenced by other things than the larval performance experiments accounted for. Things such as a greater competition with other herbivores, and more predators that may gather at these plants must be considered as well. Also, the importance of protection given by oviposition choice was demonstrated already in 1987 by Damman, who showed that protection was dominant over nutritional factors. The caterpillars he studied consistently preferred older leaves of their host even though younger leaves provided better food for growth.
Since the oviposition choice experiment demonstrated that clover was a highly ranked choice of both strains, this supports the theory of protection being one of the greatest important factors when choosing a host. The possibility to gain weight is only second to this.

EAD

From the EAD recordings in figure 8-10 there can be seen a difference between Egypt and Benin in which compounds they react to. When comparing the data from the EAG compiled in figure 11, the compounds show a significant difference after conducting a T-2 test, showing a higher sensitivity for the compounds for the Egyptian strain in (E)2-hexenal with 0,002; (E/Z)-b-ocimene (Omicene) with 0,003 and cis-3-Hexenyl acetate (Hexenyl-acetat) with a p-value of 0,004 (See appendix 3).The compounds the Egypt strain is reacting to are all strongly associated with cotton (Binyameen *et al.* 2012).

Not all adaptations develop to distinguish or compare the positive cues, but also the negative. This might be an adaptation to avoid such a plant as cotton which, according to the diet experiment, seems to hinder the larval development the most when compared to the other host plants.

Conclusions

When comparing the oviposition data there was a clear hierarchy to host plants showing that the two strains choose differently. When compared with a T2-test there could be seen significance in preference to clover between the strains, suggesting that the Egypt strain would be more attracted to clover than the Benin. After conducting a Multivaraiate Anova test there was no significance noticed between the strains preferences towards their hosts.

The curves documenting the development of *S. littorlis* larvae weight on a specific diet show a significant difference between all diets within the Benin strain after 16 days. Furthermore cotton, clover/cowpea and artificial diets in the Egypt strain also differentiated after this time (appendix 3). These curves also show a difference in development between the cowpea and cotton diets when comparing the two strains to each other, indicating that the Benin strain gains more mass during this period of time.

There can be seen a significant difference between the two strains after reaching the pupal stage on cowpea and artificial diet, implying that the Egyptian strain develops better on an artificial diet and the Benin strain on cowpea.

Since the variations between individual antenna appears to be greater than between the strains, it is difficult to compare the physiology based on the results from the GC-EAD. Instead a comparison between the mV from the EAG was made which showed significant values between the two strains concerning (E)2-hexenal, (E/Z)-b-ocimene, (Z)-3-hexenyl acetate, Nonanal, (-)-linalool and β-myrcene (appendix 5). This suggests a generally higher sensitivity towards these compounds within the Egypt strain.
The compounds the Egypt strain is reacting to are all strongly associated with cotton (Binyameen *et al.* 2012). Interpreting this as evolutionary adaptations, the strong sensitivity suggests a long progression close to cotton, where, seeing as they do not develop any greater weight based on this diet, they might very well be to avoid it as food source. This is further strengthened by the results seen in the oviposition experiment.

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Appendix

Appendix 1, Oviposition test

```
Two-sample T for Clover

C1              N   Mean  StDev  SE Mean
Benin, clover   5  21,42   4,79      2,1
Egypt, clover  13  12,62   9,59      2,7


Difference = mu (Benin, clover) - mu (Egypt, clover)
Estimate for difference:  8,80
95% CI for difference:  (1,48; 16,13)
T-Test of difference = 0 (vs not =): T-Value = 2,58  P-Value = 0,022  DF = 14



Two-sample T for Cowpea

C4              N   Mean  StDev  SE Mean
Benin, cowpea   2  20,15   7,99      5,7
Egypt, cowpea  11   14,1   10,0      3,0


Difference = mu (Benin, cowpea) - mu (Egypt, cowpea)
Estimate for difference:  6,05
95% CI for difference:  (-75,40; 87,50)
T-Test of difference = 0 (vs not =): T-Value = 0,94  P-Value = 0,518  DF = 1



Two-sample T for Cotton

C7             N  Mean  StDev  SE Mean
Benin, cotton  6  17,8   12,5      5,1
Egypt, cotton  3  21,6   11,2      6,5


Difference = mu (Benin, cotton) - mu (Egypt, cotton)
Estimate for difference:  -3,78
95% CI for difference:  (-26,68; 19,11)
T-Test of difference = 0 (vs not =): T-Value = -0,46  P-Value = 0,670  DF = 4



Two-sample T for Cabbage

C10             N    Mean  StDev  SE Mean
Benin, cabbage  2    21,0   16,7       12
Egypt, cabbage  2  11,350  0,212     0,15


Difference = mu (Benin, cabbage) - mu (Egypt, cabbage)
Estimate for difference:  9,7
95% CI for difference:  (-140,3; 159,6)
T-Test of difference = 0 (vs not =): T-Value = 0,82  P-Value = 0,564  DF = 1
```

Comparing a value of 0 is not possible, hence no test has been made on maize.

Appendix 2, Pupal weight

```
Two-sample T for Artificial

                                    SE
C14                 N   Mean  StDev  Mean
Benin, artificial   30  306,1   78,3    14
Egypt, artificial    4  473,1   48,5    24


Difference = mu (Benin, artificial) - mu (Egypt, artificel)
Estimate for difference:  -167,0
95% CI for difference:  (-239,3; -94,6)
T-Test of difference = 0 (vs not =): T-Value = -5,93  P-Value = 0,002  DF = 5



Two-sample T for Cowpea

C16             N   Mean  StDev  SE Mean
Benin, cowpea  16  169,1   48,2       12
Egypt, cowpea  16  129,9   24,5      6,1


Difference = mu (Benin, cowpea) - mu (Egypt, cowpea)
Estimate for difference:  39,1
95% CI for difference:  (11,1; 67,2)
T-Test of difference = 0 (vs not =): T-Value = 2,89  P-Value = 0,008  DF = 22



Two-sample T for Clover

C18             N   Mean  StDev  SE Mean
Benin, clover  23  216,3   63,6       13
Egypt, clover  31  199,0   50,6      9,1


Difference = mu (Benin, clover) - mu (Egypt, clover)
Estimate for difference:  17,2
95% CI for difference:  (-15,3; 49,7)
T-Test of difference = 0 (vs not =): T-Value = 1,07  P-Value = 0,290  DF = 40



Two-sample T for Cotton

C12             N   Mean  StDev  SE Mean
Benin, cotton  28  225,4   42,2      8,0
Egypt, cotton  23    217    106       22


Difference = mu (Benin, cotton) - mu (Egypt, cotton)
Estimate for difference:  8,9
95% CI for difference:  (-39,4; 57,1)
T-Test of difference = 0 (vs not =): T-Value = 0,38  P-Value = 0,709  DF = 27
```

Appendix 3, Within the weight curves (16 days)

One-way ANOVA: Benin

```
Source  DF       SS       MS      F      P
C5       3  3830641  1276880  92,91  0,000
Error   36   494758    13743
Total   39  4325399

S = 117,2   R-Sq = 88,56%   R-Sq(adj) = 87,61%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level         N   Mean  StDev  +---------+---------+---------+---------
BeninArt     10  877,5  205,8                                    (--*--)
BeninClover  10  540,8   87,0                        (--*--)
BeninCotton  10   75,0   28,7  (--*--)
BeninCowpea  10  219,0   65,1        (--*--)
                              +---------+---------+---------+---------
                              0       250       500       750

Pooled StDev = 117,2


Grouping Information Using Tukey Method

C5            N   Mean  Grouping
BeninArt     10  877,5  A
BeninClover  10  540,8    B
BeninCowpea  10  219,0      C
BeninCotton  10   75,0        D

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of C5

Individual confidence level = 98,93%


C5 = BeninArt subtracted from:

C5            Lower  Center   Upper  ----+---------+---------+---------+-----
BeninClover  -477,9  -336,7  -195,5              (---*--)
BeninCotton  -943,7  -802,5  -661,3  (---*--)
BeninCowpea  -799,7  -658,5  -517,3      (---*--)
                                     ----+---------+---------+---------+-----
                                      -800      -400         0       400


C5 = BeninClover subtracted from:

C5            Lower  Center   Upper  ----+---------+---------+---------+-----
BeninCotton  -607,0  -465,8  -324,6           (--*---)
BeninCowpea  -463,0  -321,8  -180,6              (---*--)
                                     ----+---------+---------+---------+-----
                                      -800      -400         0       400


C5 = BeninCotton subtracted from:

C5           Lower  Center  Upper  ----+---------+---------+---------+-----
BeninCowpea    2,8   144,0  285,2                          (---*--)
                                   ----+---------+---------+---------+-----
                                    -800      -400         0       400
```

One-way ANOVA: Egypt

```
Source  DF       SS      MS      F      P
C1       3  2203399  734466  33,63  0,000
Error   36   786181   21838
Total   39  2989580

S = 147,8   R-Sq = 73,70%   R-Sq(adj) = 71,51%


                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level         N   Mean  StDev  --+---------+---------+---------+-------
EgyptArt     10  691,6  246,6                            (---*--)
EgyptClover  10  433,2  147,3                  (--*---)
EgyptCotton  10   32,9   13,1  (--*---)
EgyptCowpea  10  378,5   68,4              (---*---)
                              --+---------+---------+---------+-------
                                0       250       500       750

Pooled StDev = 147,8


Grouping Information Using Tukey Method

C1            N   Mean  Grouping
EgyptArt     10  691,6  A
EgyptClover  10  433,2    B
EgyptCowpea  10  378,5    B
EgyptCotton  10   32,9      C

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of C1

Individual confidence level = 98,93%


C1 = EgyptArt subtracted from:

C1            Lower  Center   Upper    -+---------+---------+---------+--------
EgyptClover  -436,4  -258,4   -80,4              (----*---)
EgyptCotton  -836,7  -658,7  -480,7    (----*---)
EgyptCowpea  -491,1  -313,1  -135,1             (---*----)
                                  -+---------+---------+---------+--------
                                -800      -400         0       400


C1 = EgyptClover subtracted from:

C1            Lower  Center   Upper    -+---------+---------+---------+--------
EgyptCotton  -578,3  -400,3  -222,3           (---*---)
EgyptCowpea  -232,7   -54,7   123,3                   (----*---)
                                  -+---------+---------+---------+--------
                                -800      -400         0       400


C1 = EgyptCotton subtracted from:

C1           Lower  Center  Upper    -+---------+---------+---------+--------
EgyptCowpea  167,6   345,6  523,6                           (----*---)
                                  -+---------+---------+---------+--------
                               -800      -400         0       400
```

Appendix 4, Between the weight curves (16 days)

```
Two-sample T for Clover

                                SE
C26           N   Mean  StDev  Mean
BeninClover  10  540,8   87,0    28
EgyptClover  10    433    147    47


Difference = mu (BeninClover) - mu (EgyptClover)
Estimate for difference:  107,6
95% CI for difference:  (-8,5; 223,7)
T-Test of difference = 0 (vs not =): T-Value = 1,99  P-Value = 0,067  DF = 14


Two-sample T for Cowpea

                                SE
C24           N   Mean  StDev  Mean
BeninCowpea  10  219,0   65,1    21
EgyptCowpea  10  378,5   68,4    22


Difference = mu (BeninCowpea) - mu (EgyptCowpea)
Estimate for difference:  -159,5
95% CI for difference:  (-222,5; -96,5)
T-Test of difference = 0 (vs not =): T-Value = -5,34  P-Value = 0,000  DF = 17



Two-sample T for Cotton

C21           N  Mean  StDev  SE Mean
BeninCotton  10  75,0   28,7      9,1
EgyptCotton  10  32,9   13,1      4,1


Difference = mu (BeninCotton) - mu (EgyptCotton)
Estimate for difference:  42,10
95% CI for difference:  (20,40; 63,80)
T-Test of difference = 0 (vs not =): T-Value = 4,23  P-Value = 0,001  DF = 12


Two-sample T for Artificial

                             SE
C17        N  Mean  StDev  Mean
BeninArt  10   878    206    65
EgyptArt  10   692    247    78


Difference = mu (BeninArt) - mu (EgyptArt)
Estimate for difference:  186
95% CI for difference:  (-28; 400)
T-Test of difference = 0 (vs not =): T-Value = 1,83  P-Value = 0,085  DF = 17
```

Appendix 5, Compounds

Two-sample T for (E)2-hexenal

C31	N	Mean	StDev	SE Mean
Benin	11	0,297	0,117	0,035
Egypt	12	0,665	0,302	0,087

Difference = mu (Benin) - mu (Egypt)
Estimate for difference: -0,3677
95% CI for difference: (-0,5693; -0,1662)
T-Test of difference = 0 (vs not =): T-Value = -3,91 P-Value = 0,002 DF = 14

Two-sample T for Ocimene

C34	N	Mean	StDev	SE Mean
Benin	11	0,338	0,134	0,040
Egypt	12	0,578	0,196	0,057

Difference = mu (Benin) - mu (Egypt)
Estimate for difference: -0,2402
95% CI for difference: (-0,3854; -0,0949)
T-Test of difference = 0 (vs not =): T-Value = -3,46 P-Value = 0,003 DF = 19

Two-sample T for Hexenyl-acetat

C37	N	Mean	StDev	SE Mean
Benin	11	0,307	0,131	0,039
Egypt	12	0,526	0,189	0,055

Difference = mu (Benin) - mu (Egypt)
Estimate for difference: -0,2197
95% CI for difference: (-0,3606; -0,0788)
T-Test of difference = 0 (vs not =): T-Value = -3,26 P-Value = 0,004 DF = 1

Two-sample T for α-pinene

C22	N	Mean	StDev	SE Mean
Benin	11	0,412	0,154	0,046
Egypt	12	0,523	0,183	0,053

Difference = mu (Benin) - mu (Egypt)
Estimate for difference: -0,1107
95% CI for difference: (-0,2571; 0,0357)
T-Test of difference = 0 (vs not =): T-Value = -1,58 P-Value = 0,130 DF = 20

Two-sample T for Nonanal

C40	N	Mean	StDev	SE Mean
Benin	11	0,331	0,150	0,045
Egypt	12	0,563	0,240	0,069

Difference = mu (Benin) - mu (Egypt)
Estimate for difference: -0,2318
95% CI for difference: (-0,4057; -0,0580)
T-Test of difference = 0 (vs not =): T-Value = -2,80 P-Value = 0,012 DF = 18

Two-sample T for Linalool

C43	N	Mean	StDev	SE Mean
Benin	11	0,351	0,148	0,045
Egypt	12	0,605	0,241	0,070

Difference = mu (Benin) - mu (Egypt)
Estimate for difference: -0,2541
95% CI for difference: (-0,4278; -0,0804)
T-Test of difference = 0 (vs not =): T-Value = -3,07 P-Value = 0,007 DF = 18

Two-sample T for β-myrcene

C25	N	Mean	StDev	SE Mean
Benin	11	0,503	0,188	0,057
Egypt	12	0,719	0,274	0,079

Difference = mu (Benin) - mu (Egypt)
Estimate for difference: -0,2164
95% CI for difference: (-0,4200; -0,0128)
T-Test of difference = 0 (vs not =): T-Value = -2,23 P-Value = 0,038 DF = 19

Two-sample T for β-caryophyllene

C28	N	Mean	StDev	SE Mean
Benin	11	0,861	0,347	0,10
Egypt	12	0,950	0,365	0,11

Difference = mu (Benin) - mu (Egypt)
Estimate for difference: -0,089
95% CI for difference: (-0,399; 0,221)
T-Test of difference = 0 (vs not =): T-Value = -0,60 P-Value = 0,556 DF = 20

Appendix 6, Manova

General Linear Model: Maize; Cowpea; ... versus Strain

MANOVA for Strain

s = 1 m = 1,5 n = 12,5

Test DF					
Criterion	Statistic	F	Num	Denom	P
Wilks'	0,71540	2,148	5	27	0,090
Lawley-Hotelling	0,39782	2,148	5	27	0,090
Pillai's	0,28460	2,148	5	27	0,090
Roy's	0,39782				

Cluster Analysis of Observations: Maize; Cowpea; Cotton; Clover; Cabbage

Standardized Variables, Euclidean Distance, Single Linkage